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RESEARCH FROM THE COASTAL PLAIN EXPERIMENT STATION, TIFTON, GEORGIA, TO MINIMIZE AFLATOXIN CONTAMINATION IN PEANUT

C. Corley Holbrook [a]; P. Ozias-Akins [b]; P. Timper [c]; D. M. Wilson [d]; E. Cantonwine [ef]; B. Z. Guo [c]; D. G. Sullivan [g]; W. Dong [h]
[a] Crop Genetics and Breeding Research Unit, USDA-ARS, Tifton, Georgia [b] Department of Horticulture, University of Georgia, Tifton, Georgia [c] Crop Protection and Management Research Unit, USDA-ARS, Tifton, Georgia [d] Department of Plant Pathology, University of Georgia (Retired), Tifton, Georgia [e] National Environmentally Sound Production Agriculture Laboratory, University of Georgia, Valdosta, Georgia [f] Valdosta State University, Valdosta, Georgia [g] Southeast Watershed Research Unit, USDA-ARS, Tifton, Georgia [h] Department of Plant Pathology, University of Georgia, Tifton, Georgia

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C. CORLEY HOLBROOK
Crop Genetics and Breeding Research Unit, USDA-ARS, Tifton, Georgia

P. OZIAS-AKINS
Department of Horticulture, University of Georgia, Tifton, Georgia

P. TIMPER
Crop Protection and Management Research Unit, USDA-ARS, Tifton, Georgia

D. M. WILSON
Department of Plant Pathology, University of Georgia (Retired), Tifton, Georgia

E. CANTONWINE
Formerly National Environmentally Sound Production Agriculture Laboratory, University of Georgia; currently, Valdosta State University, Valdosta, Georgia

B. Z. GUO
Crop Protection and Management Research Unit, USDA-ARS, Tifton, Georgia

D. G. SULLIVAN
Southeast Watershed Research Unit, USDA-ARS, Tifton, Georgia

W. DONG
Department of Plant Pathology, University of Georgia, Tifton, Georgia

Scientists with the U.S. Department of Agriculture–Agricultural Research Service (USDA-ARS) and scientists with the University of Georgia located at the Coastal Plain Experiment Station in Tifton, Georgia, have been conducting research on aflatoxin contamination of peanut since the early 1960s. Early efforts were focused on identifying the risk factors for increased aflatoxin contamination and helped to document the importance of drought, high soil temperatures, and pod damage. Later efforts were focused on the development of screening techniques and the identification of sources of resistance to Aspergillus *colonization and/or aflatoxin contamination. This laid the foundation for a conventional resistance breeding program and has resulted in the development of peanut*

Address correspondence to Dr. C. Corley Holbrook, Crop Genetics and Breeding Res. Unit, USDA-ARS, Tifton, GA 31793. E-mail: Corley.Holbrook@ars.usda.gov

breeding lines that have high yield and low aflatoxin contamination relative to standard control cultivars. Recent research efforts include studies on the use of molecular genetic approaches to reduce aflatoxin contamination. This includes the evaluation of genetically engineered peanut and the development of molecular markers.

Keywords aflatoxin, *Arachis hypogaea*, *Aspergillus* spp., breeding, drought tolerance, peanut

Introduction

Aflatoxin contamination of peanut was first recognized as a serious problem following outbreaks of "turkey X disease" in the United Kingdom in 1960 (Lancaster et al., 1961; Sargeant et al., 1961). In that year over 100,000 turkey poults died after consuming Brazilian peanut meal. Research revealed that the meal was contaminated by *Aspergillus flavus* that was producing a toxin, and hence these toxins were named aflatoxins. Aflatoxins are human carcinogens, and acceptable levels in food are regulated for domestic and international markets. The maximum allowable level in the United States is 20 ppb and the European Union has an allowable level of 4 ppb total aflatoxins and under 2 ppb aflatoxin B_1. Scientists at the Coastal Plain Experiment Station in Tifton, Georgia, have been conducting research on aflatoxin contamination in peanut since shortly after the problem was discovered. Initially the research was focused on defining the risk factors for aflatoxin contamination. More recently the research has focused on conventional breeding and molecular genetic approaches to minimize contamination.

Risk Factors for Aflatoxin Contamination of Peanut

During the early 1960s, several researchers documented greater invasion of peanut pods by *A. flavus* when the integrity of the shell was compromised by mechanical damage, cracking, insect feeding, and fungal parasitism (Ashworth and Langley, 1964; McDonald and Harkness, 1964; Schroeder and Ashworth, 1965). At the Coastal Plain Experiment Station in Tifton, Agricultural Research Service (ARS) researchers, in collaboration with

University of Georgia researchers, subsequently began to explore the role of soilborne pests on invasion of the peanut pods by toxigenic *Aspergillus* spp. and on aflatoxin contamination. Plant-parasitic nematodes were one of the first pests to be studied. Root-knot (*Meloidogyne* spp.) and root-lesion (*Pratylenchus brachyurus*) nematodes can infect the developing pods as well as the roots of peanut. Minton and Jackson (1967) hypothesized that the pod galls and lesions caused by *Meloidogyne* spp. and *P. brachyurus*, respectively, might create access points for toxigenic fungi to infest the peanut kernel. Over the next several years, they tested this hypothesis in greenhouse, field, and microplot experiments. In a series of experiments with *M. arenaria* and *P. brachyurus*, there was no increase in *A. flavus* populations in the peanut kernel when the nematodes were present, despite moderate to severe damage to the pod (Minton and Jackson, 1967; Jackson and Minton, 1968; Bell et al., 1971). However, in a single trial of a microplot experiment with *M. hapla*, greater colonization of the kernels occurred when *A. flavus* was applied along with the nematode than when the fungus was applied alone (Minton et al., 1969). In all four studies, aflatoxin contamination of the kernels was nil to low. At this point in time, it appeared that pod damage by root-knot nematodes could occasionally lead to greater invasion of the peanut kernel by *A. flavus*, but the nematode's role in aflatoxin contamination was inconclusive.

In the early to mid-1980s, drought stress and high soil temperatures 3 to 6 weeks before harvest were shown to be the primary contributing factors to aflatoxin contamination in peanut (Hill et al., 1983; Wilson and Stansell, 1983; Blankenship et al., 1984; Sanders et al., 1985). Wilson and Gascho (1989) demonstrated that a lack of calcium can be a secondary factor in increasing aflatoxin contamination. Armed with this knowledge, Lynch and colleagues (1990) investigated the interaction between the lesser cornstalk borer (*Elasmopalpus lignosellus*), *A. flavus*, and drought. They found an increase in aflatoxin contamination of kernels in plots infested with the insect compared with control plots, and this increase only occurred under drought stress. Moreover, damaged pods had a higher incidence of *A. flavus* and aflatoxin concentrations than undamaged pods. In a follow-up study, Lynch and Wilson (1991) showed that contamination of kernels was directly related to the extent of pod injury by the lesser cornstalk borer,

with penetrated pods and partially consumed pods having greater kernel contamination by *A. parasiticus* and aflatoxin compared with uninjured or externally injured pods. Perhaps their most significant finding, however, was that external scarification of the pods by lesser cornstalk borer led to greater contamination of the kernel by *A. flavus* group fungi, though this did not lead to greater aflatoxin concentrations.

In the early 2000s, Timper and colleagues (2004) re-examined the effect of root-knot nematodes on aflatoxin contamination because the earlier research was performed before the primary risk factors for preharvest aflatoxin contamination were known. In the more recent experiments, drought was induced several weeks before peanut harvest. In treatments where *A. flavus* inoculum was added, aflatoxin concentrations were high and not affected by *M. arenaria*. However, in treatments without added fungal inoculum, aflatoxin concentrations were greater in kernels from nematode-infected plants than in kernels from uninfected plants. Toxigenic *Aspergillus* spp. are ubiquitous in nature. Adding *A. flavus* inoculum increased soil populations of the fungus, which may have masked the effects of the nematode. Similar to earlier studies, colonization of the kernels by *A. flavus* was not increased in treatments with *M. arenaria* compared with treatments without the nematode. However, in the first year of the study, there was a correlation between level of pod galling and both the colonization of the kernel by *A. flavus* and aflatoxin concentrations (Timper et al., 2004).

Research is under way to determine the mechanism by which nematodes increase preharvest aflatoxin contamination. Although it appears that pod galling can increase infection of the kernel by toxigenic *Aspergillus* spp., the contribution of root galling to aflatoxin production was not known. Nematode damage to the roots results in greater drought stress, which may result in greater aflatoxin production. Nematode infection of roots also causes physiological changes in the plant, which may increase its susceptibility to infection by toxigenic *Aspergillus* spp. or the production of aflatoxins. Recent experiments have demonstrated that root galling, even in the absence of pod galling, can increase aflatoxin contamination of the peanut kernel (Timper et al., 2007).

Conventional Breeding for Reduced Aflatoxin Contamination

Aflatoxin contamination of peanut is one of the most serious challenges facing the peanut industry (Cole et al., 1995). The development of peanut cultivars with resistance to aflatoxin contamination could serve as a valuable tool in addressing this challenge. There are two requirements for developing peanut cultivars with resistance to aflatoxin contamination. There must be genetic variation for resistance and there must be screening techniques that can be used to reliably measure this variation.

Screening Techniques

Early efforts to identify resistance to aflatoxin contamination in peanut involved laboratory screening techniques based on fungal colonization as measured by sporulation on rehydrated peanut seed. Mixon and Rogers (1973) developed the dried seed laboratory inoculation method to screen peanut genotypes for resistance to *A. flavus* invasion and subsequent sporulation. Using this technique, they identified two accessions, PI 337394F and PI 337409, that showed a high level of resistance to in vitro seed colonization by *A. flavus*. Mixon (1983a, 1983b) also developed 6 breeding lines that exhibited significant resistance to in vitro seed colonization over 4 years of testing (Mixon, 1986). Several other researchers have used Mixon and Rogers's method, or modifications of it, to screen peanut germplasm for resistance to in vitro seed colonization by *A. flavus* (LaPrade et al., 1973; Bartz et al., 1978; Mehan et al., 1981; Zambettakis et al., 1981; Tsai and Yeh, 1985).

Screening peanut germplasm for resistance to in vitro seed colonization is subject to a number of limitations. Bartz and colleagues (1978) examined 18 genotypes over 4 years and found that different seed lots of the same line did not always yield similar results unless the dates of digging, method of curing, and location of planting were the same. Mixon (1986) and Mehan and colleagues (1983) also observed significant genotype by environment interactions on resistance to in vitro seed colonization.

Despite the effects of environment and genotype by environment interactions on resistance to in vitro seed colonization, this screening technique has been successfully used to identify genotypes that exhibit resistance to in vitro seed colonization when fungal sporulation is assessed. A more serious limitation in using this technique has been poor correlations between in vitro seed colonization and field colonization and between in vitro seed colonization and field aflatoxin contamination (Blankenship et al., 1985; Kisyombe et al., 1985; Anderson et al., 1995).

Blankenship and colleagues (1985) examined four peanut genotypes previously selected as resistant to in vitro colonization. All genotypes were highly contaminated with aflatoxin when subjected to preharvest drought and temperature conditions conducive to *A. flavus* invasion and aflatoxin contamination. They suggested the need for further research to develop an accurate screening method to identify genetic resistance to preharvest aflatoxin contamination in peanut germplasm.

The in vitro screening technique also does not appear to be correlated with reduced aflatoxin contamination under postharvest conditions conducive to aflatoxin contamination. Wilson and colleagues (1977) examined the aflatoxin contamination that developed under high-humidity storage conditions in shelled and nonshelled peanuts of two genotypes with resistance to in vitro colonization, "Florunner," and a susceptible control genotype. All genotypes had appreciable levels of aflatoxin after 9 to 10 days of storage in relative humidity of 87% to 95% at 23 to 26°C.

Holbrook and colleagues (1994) developed a large-scale field screening technique that can be used to directly measure field resistance to preharvest aflatoxin contamination. This technique is based on the use of subsurface irrigation in a desert environment to allow for an extended period of drought stress in the pod zone while keeping the plant alive. In initial field tests conducted in the desert environment without subsurface irrigation, peanut plants died and the seeds rapidly dehydrated in the soil, before contamination could occur. The use of subsurface irrigation increased the mean aflatoxin contamination by over 100%, reduced the C.V. by over 50%, and reduced the percentage of escapes by over 90% in comparison with desert screening without subsurface irrigation.

A movable greenhouse system (Atlas Greenhouse Systems, Inc., Alapaha, Georgia) was developed to provide a screening site

at Tifton. Thirteen large (9.1 m wide × 25.5 m long) rainout shelters were constructed on skids. These structures can be moved in the field with tractors and are parked on the test plots for the 40 days immediately preceding harvest to provide the extended period of heat and drought stress necessary for consistent aflatoxin contamination of susceptible genotypes. They can be used with two planting dates each season. This system is being successfully used to screen for resistance to preharvest aflatoxin contamination in Tifton.

Anderson and colleagues (1996) developed a screening technique that can be used in standard greenhouse facilities. Methods of obtaining adequate drought–stress and fungal infections were developed through this series of experiments. High amounts of preharvest aflatoxin accumulation were produced by completely isolating the pod zone and restricting moisture to the root zone. The greenhouse methods developed in this research will be useful tools for identifying and studying sources of resistance to aflatoxin.

Artificial inoculation is frequently used when screening germplasm for resistance. Artificial inoculation helps to insure uniform testing conditions, which reduces the number of escapes and reduces variation in the data that could mask genetic differences. The standard method for inoculating peanut with *Aspergillus* had been a spore suspension in water applied at midbloom. This provided a high initial fungal pressure; however, soil populations of *Aspergillus* declined rapidly shortly after inoculation. Will and colleagues (1994) developed a new method using cracked corn as a carrier for the fungus. The theory behind this new method was that the corn would serve as a food source for the fungus and result in more stable fungal inoculum on the developing pods. The use of corn as a carrier resulted in significantly greater soil populations of *Aspergillus* at harvest than the use of water as a carrier. This inoculation technique should help reduce the inherent variability of preharvest aflatoxin contamination and is being used for the germplasm screening and plant breeding efforts at Tifton.

Aflatoxins were the first mycotoxins to be regulated and can be extracted and analyzed with a variety of techniques. The most suitable techniques for use with peanut have been reviewed (Wilson et al., 1998; Waltking and Wilson, 2006).

Genetic Variation

The above screening techniques were used to examine peanut germplasm for resistance to preharvest aflatoxin contamination. The first set of germplasm examined was the core collection (Holbrook et al., 1993). All accessions in the peanut core collection are first examined in a preliminary screen using five replications in a single environment. Genotypes that had low contamination levels in the preliminary screen were then examined for a second year using 10 replications in two environments. Screening of the U.S. peanut core collection resulted in the identification of 19 core accessions that showed low levels of aflatoxin contamination in multiple environments (Holbrook et al., 2009; Peanut Sci. 36. These genotypes have been entered into a hybridization program to combine the resistance with acceptable agronomic performance.

Peanut genotypes that have resistance to other fungi have been reported and are available. Holbrook and colleagues (1997) conducted a study to determine if these genotypes might also have resistance to *A. flavus* and/or aflatoxin contamination. Nine peanut genotypes with resistance to leaf spot and/or white mold were evaluated for 2 years at Tifton, Georgia, and Yuma, Arizona. Plots were subjected to late-season heat and drought stress. None of these genotypes exhibited less colonization of shells or kernels by *A. flavus* group fungi than the standard control when tested in Georgia or Arizona. Moreover, none of these genotypes showed a reduced level of aflatoxin contamination in comparison with a standard control at either location. These results indicated that the mechanisms of resistance to other fungi operating in these genotypes are not effective in providing resistance to colonization by *A. flavus* group fungi or reduced aflatoxin contamination.

Information in the literature indicates that fatty acid composition might directly or indirectly affect aflatoxin biosynthesis by *Aspergillus* spp. (Fabbri et al., 1983; Doehlert et al., 1993; Burow et al., 1997). Holbrook, Wilson, and colleagues (2000) tested seven peanut breeding lines with low linoleic composition under heat and drought stress conditions to determine if they would have reduced preharvest aflatoxin contamination. None of the lines had aflatoxin contamination significantly lower than the standard control cultivar. They concluded that the products of

the lipoxygenase pathway that have been shown to affect aflatoxin biosynthesis in vitro may not be present in sufficient quantities in developing peanut seed. However, under conditions that simulated postharvest conditions, Xue and colleagues (2003) observed an increased ability of high-oleic lines to support production of aflatoxin in comparison with normal-oleic lines. They urged special care in handling and storage of high-oleic peanut to prevent the growth of *Aspergillus* spp.

Breeding Progress

Sources of resistance to preharvest aflatoxin contamination have been crossed with high-yielding cultivars and breeding lines. Because of the low heritability for this trait, these populations are advanced to later generations using single seed descent. Initial selection is practiced on $F_{4:6}$ progeny using five replications. Selections are retested the following year using 10 replications. This procedure has been used to produce late generation breeding lines that have exhibited high relative yield and low relative aflatoxin contamination in multiple environments.

Research is ongoing to attempt to identify indirect selection tools that may be used to select for resistance to preharvest aflatoxin contamination. An indirect selection tool could be very valuable in reducing the cost of selecting for low aflatoxin contamination. Drought tolerance may serve as an indirect selection tool for resistance to preharvest aflatoxin contamination; however, conflicting results have been reported in the literature. Kisyombe and colleagues (1985) examined the colonization of seed by *A. parasiticus* in drought-stress and non-stress plots. They examined 14 genotypes including three that had been reported to have some drought tolerance. Although the drought-tolerant lines were susceptible to *A. parasiticus*, infection of two of these genotypes was not enhanced by drought stress. Mehan and colleagues (1987) and Mehan (1989) also observed that several drought-tolerant genotypes were susceptible to colonization and subsequent contamination by aflatoxin. However, Mehan (1989) observed relatively low levels of seed infection in one drought-tolerant genotype and concluded that more research was needed to determine if drought tolerance can reduce stress on pods and seeds to a level that would reduce aflatoxin contamination. The

results of Sanders and colleagues (1993) may suggest that drought tolerance will not reduce the stress on developing pods to a level that would reduce aflatoxin contamination. They observed high levels of aflatoxin in Florunner peanut grown with irrigation in the root zone and drought stress in the pod zone.

Holbrook, Kvien, and colleagues (2000) evaluated resistance to preharvest aflatoxin contamination in a set of genotypes that had been documented as having varying levels of drought tolerance (Ruckers et al., 1995) and determined the correlation of drought tolerance characteristics with aflatoxin contamination. The 20 genotypes were tested for 2 years under drought stress conditions at Yuma, Arizona, and Tifton, Georgia. Drought tolerance was very effective in reducing aflatoxin contamination in Tifton; however, it was not effective in reducing aflatoxin contamination in Yuma. They proposed that the ability of drought tolerance to serve as a mechanism to reduce aflatoxin may have been overwhelmed by the low relative humidity at the Yuma location. At Tifton, significant positive correlations were observed between aflatoxin contamination and visual stress ratings. A significant negative correlation was also observed between aflatoxin contamination and yield under drought stress conditions. Leaf temperature, visual stress ratings, and yield are less variable and cheaper to measure than aflatoxin contamination. These characteristics may be useful as indirect selection tools for reduced aflatoxin contamination.

Epidermal conductance is a measure of the loss of water vapor from leaves when stomata are closed. Cantonwine and colleagues (2006) compared epidermal conductance values of peanut genotypes with varied levels of field resistance to drought to assess the use of this measurement as a potential drought response trait in peanut. Unfortunately, the genetic variation in epidermal conductance did not appear to be large enough to be useful in our breeding program. Dong and colleagues (2002) also evaluated the use of SPAD chlorophyll meter reading (SCMR) as a possible selection criteria. No significant correlations were observed between SCMR and visual drought stress ratings or between SCMR and aflatoxin contamination. More promising results were observed in the use of ground-based remote sensing of canopy reflectance as a selection criteria for drought- and aflatoxin-resistant peanut genotypes (Sullivan and Holbrook, 2007).

Maarouf and colleagues (1999) used cowpea as a model plant to study the PLD (phospholipase D, a main enzyme responsible for the drought-induced degradation of membrane phospholipids in plants) enzymatic activity and gene expression under water stress in two cultivars, drought-tolerant and drought-susceptible, and found that PLD enzymatic activities increased when plants were exposed to water stress, the increase being much higher in the drought-susceptible cultivar than in the drought-tolerant cultivar. Guo and colleagues (2006) identified a novel peanut *PLD* gene and studied the *PLD* gene expression under drought stress using four drought-tolerant or -sensitive peanut lines. Northern analysis showed that *PLD* gene expression was induced faster by drought stress in the drought-sensitive lines than the drought-tolerant lines. These results suggest that peanut *PLD* may be involved in drought sensitivity and tolerance responses, and the gene expression may be useful as a tool in germplasm screening for drought tolerance.

Timper and colleagues (2004, 2007) have demonstrated that peanut root-knot nematode (*M. arenaria*) can increase aflatoxin contamination of peanut kernels when the plants are subjected to drought stress during pod maturation. Holbrook and colleagues (2008) have recently released Tifguard, a nematode-resistant cultivar with excellent resistance to tomato spotted wilt virus (TSWV). Research is ongoing to determine if this cultivar can be used as a tool to reduce aflatoxin contamination in the southeastern United States.

Molecular Genetic Approaches for Reducing Aflatoxin Contamination

Resistance to aflatoxin contamination in peanut is complex and most likely multigenic. The potential to enhance genetic resistance through genetic engineering is great provided that major effect genes can be identified and introduced into the crop. Genetic engineering of peanut was first reported in the early 1990s (Ozias-Akins et al., 1993) and can be accomplished by free DNA delivery methods such as microprojectile bombardment or by *Agrobacterium*-mediated delivery of foreign DNA (Ozias-Akins and Gill, 2001; Ozias-Akins, 2007). Transformation via *Agrobacterium tumefaciens* is more genotype dependent than

microprojectile bombardment, and only the latter has thus far been successful for runner peanut varieties commonly grown in the southeastern United States. Transformation efficiencies are relatively low compared with most crops, although adequate numbers of low copy insertions can be obtained in one person-year per construct.

Host plant resistance to aflatoxin contamination can be approached from multiple directions, for example, reducing fungal contamination and/or reducing aflatoxin biosynthesis. Aflatoxin is a secondary metabolite of the fungi *Aspergillus flavus* and *A. parasiticus*, and its synthesis may be triggered by host stress signals. It is known that irrigation of peanuts can essentially eliminate aflatoxin contamination and that drought stress will prompt its synthesis; therefore, alleviating stress with more drought-tolerant cultivars may be one means to reduce aflatoxin contamination. Drought tolerance also is a complex trait and mechanistically diverse; therefore, the use of genetic engineering where single major effect genes are overexpressed has not yet resulted in the release of a transgenic drought-tolerant cultivar. However, in peanut, it is known that drought also promotes insect feeding on peanut pods, in particular by the lesser cornstalk borer (LCB). LCB damages peanut pods by boring holes through the pericarp or by scarifying its surface, and provides access points for the fungus, which also is carried by the insect. This lepidopteran insect pest is known to be susceptible to certain insecticidal crystalline proteins from the soil bacterium *Bacillus thuringiensis*; therefore, expression of CryIA(c) in peanut was tested for its efficacy in controlling the insect pest and reducing aflatoxin contamination. A synthetic gene under the control of a CaMV 35S promoter was introduced into peanut cultivar MARC I and was shown in vitro to dramatically reduce leaf feeding by LCB (Singsit et al., 1997; Ozias-Akins et al., 2002). Pod damage in the field also was significantly reduced by expression of CryIA(c) and correspondingly, aflatoxin contamination was reduced (Ozias-Akins et al., 2002).

Preventing entry of the fungi into peanut pods through insect control can likely impact, but not solve the aflatoxin contamination problem. Further strategies to reduce fungal growth once entry is gained include the testing of putative antifungal peptides and proteins. Two examples are a non-heme chloroperoxidase

(CPO) from *Pseudomonas pyrrocinia* and an anti-apoptotic gene from human. Leaf extracts from transgenic tobacco transformed with the CPO gene had antifungal activity against *A. flavus* in in vitro assays (Rajasekaran et al., 2000). Similar results were observed with transgenic peanut expressing CPO (Niu et al., 2002). The anti-apoptotic gene, Bcl-xl, previously was shown to confer broad-spectrum resistance to fungal necrotrophs in tobacco as well as to viral and abiotic (herbicide) stresses (Dickman et al., 2001; Chen and Dickman, 2004). Comparable results for herbicide tolerance were observed in transgenic peanut expressing Bcl-xl, although expression of Bcl-xl probably had a detrimental effect on plant reproduction, and the gene eventually was silenced in all initially expressing lines (Chu et al., 2008). Insufficient plant material, therefore, was available for aflatoxin testing.

In the event that aflatoxin contamination cannot be completely controlled by reducing fungal invasion and growth, the possibility exists to control the induction of aflatoxin biosynthesis by manipulating host factors that signal stress. We know that lipid peroxidation products, specifically oxylipins, can affect *Aspergillus* spp. development and aflatoxin production (Burow et al., 1997). Since lipoxygenase enzymes play a large role in oxylipin production, their differential expression in response to stress (biotic and abiotic) has been extensively studied in several plants and partially elucidated in peanut. The lipoxygenase gene family is complex and many questions remain regarding the response of seed-expressed LOX genes to biotic and abiotic stresses. Expression of peanut seed LOX family members in response to *A. flavus* currently is under intensive study.

Liang and colleagues (2005, 2006) investigated the possible association of storage proteins with resistance to aflatoxin contamination and used total protein profiles to identify possible proteins as resistance "markers." It was interesting that the isoforms of β-1,3-glucanase were revealed differently in different peanut genotypes as a result of infection of *A. flavus*. In the untreated control peanut seeds, the base-lines of endogenous β-1,3-glucanase were similar in all tested genotypes. In the seeds inoculated with *A. flavus* in vitro the activities of β-1,3-glucanase were increased significantly in the resistant genotypes in comparison with the susceptible genotypes. TLS analyses of the hydrolytic

products from the reaction mixtures demonstrated the presence of the enzyme β-1,3-glucanase. The peptide sequences of the protein corresponding to the band of β-1,3-glucanase isoforms from native PAGE have homology to conglutin, a storage protein in peanut seeds. Peanut total protein profiles have revealed polymorphic markers and genetic variation among peanut genotypes and subspecies. Guo, Liang, and colleagues (2008) identified polymorphic protein bands among tested peanut genotypes by profiling peanut total seed proteins with SDS-PAGE and 2-D PAGE. One peanut line (GT-C9) was identified lacking several seed storage proteins. These polymorphic protein peptides distinguished by 2-D PAGE need to be studied further for possible marker application. Identification of authentic resistance-related protein markers and/or genes could lead to the enhancement of antifungal activities in peanut seeds through marker-assisted selection in breeding, or by direct up- or down-regulation of the target genes using genetic engineering.

Genomics research on expressed sequence tags (ESTs), microarray technologies, and whole genome sequencing should provide important insight on genes for resistance to *Aspergillus* spp. and/or aflatoxin contamination. In spite of a continuous decrease in DNA sequencing costs, it is improbable that many large plant genomes, such as peanut, will be sequenced in the near future. However, partially sequencing of large numbers of expressed genes (ESTs) can deliver substantial amounts of genetic information. Notable research progress has been made recently in development of peanut ESTs (Luo, Dang, Guo, et al., 2005; Guo, Chen, et al., 2008a). Microarray technology has empowered researchers to conduct genome-wide or global gene expression analysis. The ability to study changes in the expression of thousands of genes simultaneously has made it possible to associate genes with predictive functions or specific physiological conditions. Studies with peanut have demonstrated the power of EST and microarray technologies (Luo, Dang, Guo, et al., 2005; Luo, Dang, Holbrook, et al., 2005; Luo, Liang, et al., 2005; Guo et al., 2005; Guo, Chen, et al., 2008). Genes identified through gene expression analyses in these studies might be associated with drought tolerance, resistance to infection of *Aspergillus flavus*, and aflatoxin contamination.

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